

Claims

1. A method of identifying a polymorphic receptor having altered signaling, comprising the steps of:

5 a) cotransfecting a first host cell with a reporter construct and an expression vector, said reporter construct comprising a response element and a promoter operably linked to a reporter gene, said response element being sensitive to a signal induced by said receptor, and said expression vector comprising a promoter operably linked to a candidate receptor having a genetic polymorphism;

10 b) cotransfecting a second host cell with said reporter construct and a negative control vector; and

c) measuring the level of expression of said reporter construct in said first host cell and said second host cell, an increased or decreased level of expression in the first host cell compared to the second host cell identifying said candidate
15 receptor as a polymorphic receptor having altered signaling.

2. The method of claim 1, wherein said signaling is ligand dependent signaling.

20 3. The method of claim 1, wherein said signaling is ligand independent signaling.

4. The method of claim 1, wherein said polymorphic receptor having altered signaling is a polymorphic receptor having an increase or decrease in basal
25 signaling.

5. The method of claim 1, wherein said polymorphic receptor having altered signaling is a polymorphic receptor having an increased or decreased sensitivity to ligand induced signaling.

5 6. The method of claim 1, wherein said polymorphic receptor having altered signaling is a polymorphic receptor having increased or decreased potency.

7. The method of claim 1, wherein said polymorphic receptor having altered signaling is a polymorphic receptor having an absence of signaling.

10 8. The method of claim 1, wherein said polymorphic receptor having altered signaling is a G protein-coupled receptor.

15 9. The method of claim 8, wherein said G protein-coupled receptor is coupled to a G protein selected from the group consisting of $G\alpha_q$, $G\alpha_s$, and $G\alpha_i$.

10. The method of claim 1, wherein said polymorphic receptor having altered signaling is a single transmembrane receptor.

20 11. The method of claim 10, wherein said single transmembrane receptor is an erythropoietin receptor.

12. The method of claim 1, wherein said polymorphic receptor having altered signaling is a nuclear receptor.

13. The method of claim 12, wherein said nuclear receptor is a steroid hormone receptor.

14. The method of claim 1, wherein said polymorphic receptor having
5 altered signaling is further screened for an alteration in ligand induced response.

15. The method of claim 14, wherein said ligand is a drug.

16. The method of claim 1, wherein, in step (c), the basal level of
10 expression of said reporter construct is measured in said first host cell and said second host cell, and an increased basal level of expression in said first host cell compared to said second host cell identifies said polymorphic receptor as a constitutively active receptor.

17. The method of claim 1, wherein said measuring is accomplished using
15 a transcriptional reporter assay.

18. The method of claim 1, wherein said response element is selected from the group consisting of the somatostatin promoter element, the serum response
20 element, and the cAMP response element.

19. The method of claim 1, wherein said receptor is naturally occurring.

20. The method of claim 1, wherein said polymorphic receptor is a
25 constitutively active receptor.

21. The method of claim 1, wherein said polymorphic receptor is a hypersensitive or hyposensitive receptor.

22. The method of claim 1, wherein said polymorphic receptor is a non-functional receptor.

23. A method of identifying a G protein-coupled receptor with altered signaling, said method comprising:

a) co-transfecting a first host cell with:

- i) a reporter construct, said reporter construct comprising a G protein response element and a promoter operably linked to a reporter gene,
- ii) a first expression vector, said first expression vector comprising a promoter operably linked to a candidate G protein-coupled receptor, and
- iii) a second expression vector, said second expression vector comprising a promoter operably linked to a chimeric G protein, wherein said chimeric G protein is capable of receiving a signal from said candidate G protein-coupled receptor and increasing the expression of said reporter construct;

b) co-transfecting a second host cell with said reporter construct, said second expression vector, and a negative control vector; and

c) measuring the level of expression of said reporter construct in said first host cell and said second host cell,

wherein an increased or decreased level of expression in the first host cell

compared to the second host cell identifies said candidate receptor as a G protein-coupled receptor with altered signaling.

24. The method of claim 23, wherein said chimeric G protein comprises a
5 G protein with the C-terminal 3 amino acids changed to those of another G protein.

25. The method of claim 23, wherein chimeric G protein is selected from
the group consisting of Gq5i, Gq5o, Gq5z, Gq5s, Gs5q, and G13Z.

10 26. The method of claim 23, wherein said reporter construct is selected from the group consisting of a luciferase construct, a beta-galactosidase construct, and a chloramphenicol acetyl transferase construct.

15 27. The method of claim 23, wherein reporter construct is a luciferase construct.

20 28. The method of claim 23, wherein said response element is selected from the group consisting of the somatostatin promoter, the serum response element, and the cAMP response element.

25 29. The method of claim 23, wherein said G protein coupled receptor is selected from the group consisting of a constitutively active receptor, a hypersensitive receptor, a hyposensitive receptor, a non-functional receptor, a silent receptor, and a partially silent receptor.

30. The method of claim 23, wherein said G protein-coupled receptor is coupled to a G protein selected from the group consisting of Gαq, Gαs, Gαi, and Go.

5 31. The method of claim 23, wherein said signaling is ligand dependent signaling.

32. The method of claim 23, wherein said signaling is ligand independent signaling.

10 33. The method of claim 23, wherein said G protein coupled receptor is further screened for an alteration in a response induced by a ligand.

15 34. The method of claim 33, wherein said ligand is selected from the group consisting of a drug, an agonist, an antagonist, and an inverse agonist.

35. A method of identifying a receptor having decreased signaling activity, comprising the steps of:

20 a) cotransfecting a first host cell with a reporter construct and an expression vector, said reporter construct comprising a response element and a promoter operably linked to a reporter gene, said response element being sensitive to a signal induced by said receptor, and said expression vector comprising a promoter operably linked to a candidate receptor;

25 b) cotransfecting a second host cell with said reporter construct and a negative control vector; and

c) measuring the level of expression of said reporter construct in said first

host cell and said second host cell, a decreased level of expression in the first host cell compared to the second host cell identifying said candidate receptor as a receptor having decreased signaling activity.

- 5 36. The method of claim 35, wherein said receptor has no signaling activity.

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